FLUORIDE VARNISH CONCENTRATION GRADIENT EFFECTS MEASURED BY QUANTITATIVE LIGHT FLUORESCENCE

by

Chad Owen Hazelrigg

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Margherita Fontana

George K. Stookey

Charles O. Hazelrigg

Susan Zunt

Jeffrey A. Dean
Chair of the Committee

Date_____

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INTRODUCTION

Although now considered the standard of care in most of Europe, Scandanavia, and Canada for over the last 25 years, fluoride varnishes, when used as professional applications of topical fluoride, are not as popular in the US. Fluoride varnish is not approved by the FDA as an anti-caries topical fluoride, but rather as a cavity liner and root desensitizer. However, over half of the dentists using these varnishes apply them as topical fluoride treatments. A multitude of studies have shown the anti-caries effects of fluoride varnishes to be in the range of 20 percent to 75 percent.[1-6] Two of the four varnishes sold on the American market today are sold in 10-ml tubes of 5.0-percent sodium fluoride varnish (Duraphat and Duraflor). Pilot studies have shown that a separation of contents within these varnish tubes exists.[7] Other studies have shown that although both Duraphat and Duraflor have similar formulas, Duraphat fluoride varnish provides a greater release and uptake of bioavailable fluoride then Duraflor.[8]

The early caries process typically produces a white spot lesion (area of demineralization) on the enamel surface of a tooth. It is believed that by detecting early areas of demineralization prior to frank cavitation, specific caries regimens can be initiated to allow the lesion to be arrested or even reversed. In an effort to detect these lesions, promising research is currently being conducted in the use of quantitative light-induced fluorescence (QLF).

The purpose of the current study is four-fold: 1) to measure the fluoride concentration gradient in 10-ml tubes of fluoride varnish, based on the resting position of the tube prior to use; 2) to compare a varnish's concentration gradient to its ability to

inhibit caries in an artificial caries environment; 3) to compare and contrast the fluoride concentration gradients and caries inhibition properties of three fluoride varnishes on the American market (Duraphat, Duraflor, and CavityShield); and finally 4), to determine if QLF can detect differences in lesions developed when exposed to an artificial caries environment and fluoride varnish.

HYPOTHESES

1. There will be a wide fluoride concentration gradient in a given 10-ml tube of fluoride varnish, based on the resting position of the tube prior to use.

2. This fluoride concentration gradient will significantly impact the varnishes' ability to inhibit *in vitro* caries formation.

3. All brands of fluoride varnish (Duraphat, Duraflor, and CavityShield) will be similar in fluoride concentration gradient and caries inhibition properties.

4. QLF will be able to detect demineralized and remineralized incipient lesions.

REVIEW OF LITERATURE

FLUORIDE VARNISH

As caries susceptibility in a population decreases, public health preventive measures require regular review and modification to meet current and predicted disease levels. The clinical efficacy of fluoride as a caries prevention medicament has been well demonstrated for decades. Topical fluorides have been available for several decades and have shown positive results as anti-caries agents. Topical fluorides are available in several forms: fluoride-containing dentifrices, topical fluoride gels, rinses, and varnishes. The use of a varnish as a vehicle for topical application of fluoride is intended to prolong the period of contact with the enamel surface. The amount of fluoride permanently retained in the enamel is increased, enhancing the formation of fluoridated hydroxyapatite and reducing the solubility of enamel in acid.[9]

The first commercial fluoride varnish product was introduced in 1964 by Schmidt, under the trade name Duraphat (Woelm Pharma Co., Eschwege, Germany). Duraphat contains 5.0-percent sodium fluoride or 2.26-percent weight fluoride (22.6 mg fluoride/ml) in a viscous neutral colophonium base. In 1975, a second fluoride varnish system was introduced by Arends and Schuthof. Under the trade name Fluor Protector (Vivadent, Schaan, Liechtenstein), this product is a polyurethane-based varnish containing 0.1-percent fluoride (1.0 mg fluoride/ml) in the form of fluorsilane (0.9-percent weight).[10] Since then, the formulation of fluoride varnish has not changed very much. In fact, not only are these two products still used today, but only two more varnishes have been marketed since.

Although now considered to be the standard of care in most of Europe, Scandanavia, and Canada for over the last 25 years, fluoride varnishes, when used as a professional application of topical fluoride, are not as popular in the US.[11]

In 1994, fluoride varnishes were approved by the FDA as cavity liners and desensitizing agents. However, over half of the dentists using these varnishes apply them as topical fluoride treatments. The University of North Carolina Pediatric Dentistry Department has permanently replaced all other topical fluoride treatments with fluoride varnish. In fact, fluoride varnishes are now a Medicaid-covered service in the states of Washington and North Carolina.[12]

Four fluoride varnishes exist on the American dental consumer market. Two of these varnishes are sold in 10-ml tubes of 5.0-percent sodium fluoride in a resin-based solution: Duraphat (distributed in the US by Colgate Oral Pharmaceuticals, Canton, MA) and Duraflor (manufactured by Medicom, Montreal, Canada, and distributed in the US by Medicom, Buffalo, NY). Duraflor is similar in formulation to Duraphat, with the exception that it contains xylitol, an artificial sweetening agent. Fluor Protector (distributed in the US by Ivoclar, North America, Amherst, NY) is a polyurethane-carried, 0.1-percent difluorosilane fluoride varnish and is sold in single-dose vials of 0.4 ml (0.4 mg fluoride). The latest addition into the fluoride varnish marketplace is CavityShield (Omnii Products, West Palm Beach, FL). Like Duraphat and Duraflor, CavityShield is a 5.0-percent sodium fluoride (22.6 mg fluoride ion/ml) varnish in a resinous base. However, unlike Duraphat and Duraflor, CavityShield is a unit-dosed fluoride varnish. CavityShield is available in two doses (depending on the number of teeth to be treated): a 0.25 ml (12.5 mg NaF) package or a 0.4 ml (20 mg NaF) package.

As a result, each patient will be able to receive a controlled amount of fluoride (preventing over-application), reducing the chance of over-ingestion and fluoride toxicity.

Before varnish placement, it is recommended by manufacturers to perform a dental prophylaxis. However, Seppa[13] shows that this step is not necessary and that a toothbrush prophylaxis may take the place of a rubber cup prophylaxis. Thorough drying of the teeth is not required prior to placement, because the fluoride varnishes set in the presence of moisture. Rubber dam isolation is not necessary, but cotton roll isolation is recommended to help keep teeth dry. Next, the varnish is applied directly to a tooth (or teeth) at a volume recommended by the manufacturer (usually 0.3 to 0.5 ml) using a cotton-tipped applicator or brush. It is recommended that fluoride varnish not be placed onto soft tissue. The varnish will set in a few seconds, leaving a fluoride-rich layer adjacent to the enamel surface. While fluoride varnishes are intended to remain in very close proximity to enamel after placement, they are not intended to adhere permanently. Application time varies from one to four minutes, depending on the number of teeth to be treated. Manufacturers recommend that the varnish remain in contact with the tooth for at least two hours before tooth brushing and eating. Microscopic evaluations of the enamel surface by Sovari et al.[14] have shown that small blocks of varnish remain attached to enamel even after *in vitro* demineralization challenge and sonication. Arends and Schuthof[15] found that fluoride varnish produces a permanent deposit of fluoride not only at the surface but also at depths of 50 μm or more. They believed this was due to a longer effective time for reaction with the hydroxyapatite in the enamel, which allowed the fluoridated surface and subsurface enamel to serve as reservoirs for fluoride release.

Once the varnish sets, the fluoride released from the varnish may be released at a slower and more continuous rate than other topical fluoride agents.

In order for a fluoride varnish to maintain its caries preventive effect, it will eventually need to be reapplied. The frequency of varnish application is best determined based on individual caries risk. A semi-annual application has been tested most often.[16-18] Clinical trials with Duraphat by Seppa[19] testing four applications per year showed a wide range of caries-preventive efficacy with no differences compared with a semiannual application. Also, intensive treatment protocols using three applications of Duraphat in one week per year (over three[9] and four[20] years) showed caries reductions of 46 to 67 percent in proximal surfaces. Significant reductions in caries rates were also demonstrated when fluoride varnish was applied at least semi-annually versus a single yearly application.[21,22] Logically, a high-risk patient should receive more frequent applications than a low-risk child. However, Eklund et al.[23] suggest that dentists are not using topical fluorides selectively. The authors suggest that the majority of dentists among those studied do not make decisions to use topical fluoride on an individual patient basis (i.e., some never use it; some use it at every other recall, and most use it at every recall).

Over the last 25 years, the fluoride uptake *in vitro* and *in vivo*, the acid resistance, and caries-preventing effect of fluoride varnishes have been investigated in laboratory, animal and human experimental studies.[10] Laboratory investigations and *in vivo* experimental studies have shown that varnishes supply fluoride more efficiently than other topical agents. Axelsson et al.[24] showed that fluoride varnish gave a significant caries reduction compared with fluoride rinsing. In a study by Tewari et al.[1] Duraphat

was seen to reduce dental caries by 70 to 75 percent, when compared to APF treatment, which had a caries reduction rate of 32 to 35 percent. Use of a topical sodium fluoride gel was found to have a caries reduction rate of 20 to 24 percent. Seppa et al.[25] compared Duraphat varnish with APF gels in children at a high risk of developing caries. The investigators found greater, but not statistically significant, efficacy of the varnish. In a series of studies by Bravo et al.[26-28] the efficacy of dental sealants versus Duraphat in preventing occlusal decay was studied. Although the efficacy of Duraphat in preventing occlusal decay was about 63 percent, it was shown that dental sealants had a greater efficacy of around 90 percent.

A plethora of clinical trials using fluoride varnish (especially Duraphat) on permanent teeth have been performed, producing conflicting results. Some clinical studies[1,2] have shown a reduction in caries as great as 75 percent, while the results of other studies[3,4] have been essentially negative. Some studies[5,6] report caries reduction rates of 30 percent to 40 percent. More recently, Autio and Courts[29] found that fluoride varnish application is effective in reversing and arresting active enamel lesions and therefore reduces the need for restorative intervention.

A few studies have been performed to examine the caries-reducing rate of fluoride varnish in primary teeth. Holm[30] performed a study to determine the efficacy of fluoride varnish in preschool children. He found that varnish reduced caries incidence by 44 percent. Two other studies[4,31] found fluoride varnish to have little efficacy against caries. Finally, a study by Weinstein[32] that involved children ages 12 to 24 months demonstrated that fluoride varnish reduced enamel demineralization by 21 percent to 35 percent and reversed decalcification of enamel by 51 percent.

Lately, there has been a question regarding the homogeneity of fluoride varnishes, and whether sodium fluoride may separate out of solution. Both Duraphat and Duraflor are sold in 10-ml tubes. According to their manufacturers, fluorides that are dispensed from 10-ml tubes are said to contain 14 to 40 doses of 0.75 ml to 0.25 ml each. Omnii Products [7] has recently been investigating these 10-ml tubes of varnish. In several pilot studies, entire 10-ml tubes of both Duraphat and Duraflor were dispensed into glass vials. During the dispensing, both brands of varnish showed a marked color change from the start of the tube to the finish. These vials with 10-ml of varnish were allowed to sit for 24 hours. Within a day, a white sediment layer was discovered at the bottom of the vials. A study is currently underway at the University of Florida to investigate fluoride concentration gradients in 10-ml tubes of fluoride varnish. It is speculated that a fluoride concentration gradient exists throughout a 10-ml tube of fluoride varnish, and that perhaps the NaF separates out of solution, resulting in a heterogenous mixture within the tube. How long and in what position a 10-ml tube rests could affect how much NaF is actually dispensed per dose of varnish.

It is then reasonable to conclude that if a fluoride concentration gradient exists within these tubes, the varnishes' ability to inhibit *in vitro* caries will also be affected. However, Seppa et al.[33] investigated the effect of reducing the amount of fluoride in a fluoride varnish on its clinical efficacy. The authors found that no difference in clinical efficacy was noted when a 2.26-percent NaF varnish was compared with a 1.13-percent NaF varnish. The authors conclude that further studies on a less concentrated varnish are indicated, especially regarding the use of Duraphat in children. To date, no studies have

been conducted to find a threshold level of NaF concentration that will make a fluoride varnish more clinically effective.

Although both Duraphat and Duraflor are 5.0-percent NaF varnishes with similar formulas, Joziak et al.[8] concluded from an *in vitro* study that Duraphat fluoride varnish provides a greater release and uptake of bioavailable fluoride than Duraflor cavity varnish. Whether entire tubes of Duraphat and Duraflor have concentration gradients and different caries inhibition properties has not been tested.

Fluoride varnishes contain the highest fluoride concentration of any vehicle. In fact, Duraphat, Duraflor, and CavityShield all contain 2.26 mg of fluoride ion per ml, which equates to 22,600 ppm of fluoride ion. Therefore, the risk of toxicity following ingestion clearly exists, especially in the pediatric population. Ekstrand et al.[34-36] studied the plasma fluoride concentration and urinary fluoride excretion in four children (ages 4, 5, 12, and 14) following application (from 2.3 mg to 5.0 mg of fluoride given) of Duraphat varnish. The levels of plasma fluoride concentration were found to be similar to those found after brushing with a fluoridated toothpaste and considerably less than those reported for APF gels. Urinary fluoride concentration 12 hours after application was found to be well below the toxic dose. The probable toxic dose for fluoride ingestion is around 5 mg fluoride/kg. However, Cameron and Widmer[37] report that gastrointestinal symptoms were noted following ingestion of 3.0 to 5.0 mg fluoride/kg in young children. The authors also say that fatalities in children have been reported at doses of 16 mg fluoride/kg. A number of topical preparations could provide such levels for young children.

QUANTITATIVE LIGHT-INDUCED FLUORESCENCE

The traditional view of caries is mainly a progressive degradation process that can be controlled primarily through surgical preparation of both cavitated and non-cavitated tooth surfaces and the placement of restorations. However, there is recent evidence that an increasing number of US dentists are adopting the principles of managing caries as a controllable infectious disease.[38] A large number of dentists are implementing early caries detection techniques to arrest the early caries process to avoid the less conservative option to surgically intervene. Anusavice[38] lists six main benefits of early caries detection: 1) increased potential to remineralize demineralized tooth surfaces; 2) decreased risk of progression to the cavitation stage; 3) reduced probability for future tooth sensitivity that is associated with deeper carious lesions; 4) reduced treatment cost associated with premature surgical intervention; 5) maintenance of natural occlusion; and 6) preservation of the natural esthetic appearance of tooth enamel.

Conventional diagnosis of dental caries involves visual examination with a mouth mirror, tactile examination with a sharp dental explorer, and radiographic examination with a bitewing radiograph. The mouth mirror is used to observe changes in demineralization, detect surface smoothness or roughness, color change, and pitting in enamel. Visual diagnosis of occlusal caries typically has a very low sensitivity and a high specificity. Sensitivities were found to be low enough that only 20 percent to 48 percent of caries present is detected visually. [39-41]

A number of reports have demonstrated that probing teeth with a sharp explorer may cause damage to newly erupted teeth or create a cavity at the site of a superficial

caries lesion. In a study by Ekstrand et al.[42] newly erupted third molars were probed. After extraction, the teeth were histologically analyzed in the fissure regions. In the probed teeth, 60 percent of the fissures showed signs of tissue loss, significantly greater than the 7.0 percent seen in the control group. A study by vanDorp et al.[43] showed that the probing accelerated the rate of subsequent caries progression in a laboratory study with artificial fissures with incipient caries. Yassin[44] reported that lesions were converted into cavities upon probing and that the size of the defect related to the pressure force. One other potential drawback to the use of an explorer for caries detection is the inoculation of bacteria from infected sites to non-infected sites.

Radiography used to detect caries has been with dentistry for quite a long time now. And still today, the bitewing view described by Raper[45] has an important role in the detection of approximal caries. However, Lussi et al.[46] showed that with bitewing radiography, it is not possible to detect early lesions confined to enamel. On the other hand, radiography increases the low sensitivity of clinical diagnosis and can be useful in the detection of occlusal dentinal lesions.[47]

Anusavice[38] lists several goals of early caries detection. They include: 1) primary lesion detection in the outer 200 μm of smooth surface enamel; 2) primary lesion detection in the outer 200 μm of enamel in smooth surface pit and fissure areas; 3) primary lesion detection in occlusal pit and fissure areas with the outer half of enamel; 4) reduction of false positive diagnoses of caries lesions that lead to unnecessary surgical intervention; 5) more rapid assessment of remineralization efficacy; and 6) identification of high-risk patients at earlier ages. Because the width of an enamel lesion at the tooth surface controls to a great extent the width of a caries lesion in dentin, it is important to

control the progression of early enamel lesions to prevent the initial demineralization of dentin.[48]

Fluorescence, reflectance, electrical conductance or impedance, and ultrasound transmittal properties of enamel can become altered during demineralization. Many investigators have explored the use of new technologies for detection of early lesions based on these changes that occur in dental enamel during demineralization-remineralization processes.[49] According to Verdonschot et al.,[50] quantitative methods such as quantitative light-induced fluorescence (QLF), electrical conductance measurements (ECM), and quantitative fiber-optic transillumination (FOTI) have shown the highest correlation with lesion depth and are more suitable to monitor small changes in lesions over time.

Several laboratory and pre-clinical investigations by Indiana University's Oral Health Research Institute[51-56] suggested that quantitative light-induced fluorescence was the most fully developed of these methods relying on changes in optical properties of enamel. This method also provided the opportunity to quantify changes in the extent of carious lesions over time.

The QLF system (Inspektor Research Systems, the Netherlands) has several advantages over traditional techniques for detecting and monitoring incipient lesions over time. For example, no aqueous medium is needed to wet the teeth in order to examine the lesion. Also, the teeth do not need to be extracted and sectioned in order to examine lesion progression. Finally, QLF can be done *in vivo*.

QLF was designed to measure the loss of fluorescence of a carious lesion by illuminating the tooth with a beam of light (wavelength = 290 to 450 nm). This light may

be absorbed by chromophores in the enamel and dentin causing visible fluorescence. Carious lesions have a lower number of chromophores when compared with sound teeth, and thus, there is less fluorescence. Therefore, carious lesions appear darker than sound enamel.[57] This technology has been developed into an intra-oral light fluorescence system that uses QLF to assess the baseline fluorescence and longitudinal change in fluorescence in early enamel lesions *in vivo*.[58] The system currently uses an arc lamp, filtered to a small band (370 +/- 80 nm), and a camera control unit, which is connected via a liquid light guide and electrical cable to a hand-held intra-oral camera. The image of the area can then be saved and analyzed on a computer. The system integration and software are similar to the laser-based version of QLF.[59]

Clinically, QLF was used by Tranaeus et al. [60] to compare the efficacy of fluoride varnish and professional tooth-cleaning for remineralization of white spot lesions in caries-active adolescents. In this study, adolescents aged 13 to15 years and having two white spot lesions each were divided into two groups. One group had their white spots treated with fluoride varnish once every six weeks for six months. The other group received a professional tooth-cleaning once every six weeks for six months. Using QLF, it was shown that the fluoride varnish group showed decreases in the lesion areas, whereas the lesion areas in the professional cleaning groups did not significantly change. It was concluded that in high-risk patients, repeated applications of fluoride varnish had a favorable effect on the remineralization of white spot lesions, as measured after only six months. The study also confirmed that QLF was an appropriate method for evaluating caries preventive measures in short-term studies.

Patients undergoing orthodontic treatment with fixed appliances are at an increased risk for caries. Demineralization, with white spot formation, is a relatively common sequela. In order to test the application of QLF to longitudinal, *in vivo* assessment of caries lesions, a study was conducted by Al-Khateeb et al.[61] on orthodontic patients after completion of fixed appliance therapy. Immediately after bracket removal, white spot lesions were identified and measured with QLF. Caries preventive measures were intensified. After one year of these measures in place and monthly QLF measurements, lesion areas decreased, indicating some remineralization. It was concluded that the method would have general clinical application in monitoring preventive outcomes in patients at risk for caries.

Several drawbacks for QLF-incipient lesion diagnosis exist. Two such drawbacks are the inability of QLF to detect interproximal lesions and secondary caries formation in dentin. It is also noted that the degree of wetness of enamel will result in varying degrees of fluorescence.[62] When a tooth dries out, its fluorescence becomes stronger. It is very important that the wetness of teeth is kept at a controlled level while using QLF measurements in an experiment.

CONFOCAL MICROSCOPY

Researchers have examined carious lesions and the effectiveness of interceptive treatments with the use of confocal microscopy.[63,64] Specimens can be viewed with confocal microscopy by sectioning the tooth and then hydrating the tooth with a fluorescent medium (example: Rhodamine B). Gonzalez-Cabezas et al.[65] found that the use of confocal laser scanning microscopy is an effective technique for measuring *in vitro*

mineral changes in dental tissues. Confocal microscopy operates on the principle that demineralized tooth structure contains larger pores than sound tooth structure. These pores can be penetrated with a dye that will differentially fluoresce, depending on the amount of dye present. Greater demineralization causes the tooth structure to become more porous and allows more dye penetration. Pore volume is the volume of fluid that has penetrated these pores of the tooth. An increase in pore volume may indicate increased demineralization. A decrease in pore volume may indicate less demineralization, or in some cases, a type of remineralization process.

Fluorescence of the Rhodamine B dye occurs when the dye is illuminated with an ion argon laser using a 488-nm excitation wavelength. Benn and Watson[66] used Rhodamine blue as the dye to measure the depth of natural carious lesions and found a correlation between lesion size of confocal images and backscattered electron images. Attenuating the output from the laser using a range of neutral density filters to achieve the 488-nm excitation wavelength controls the amount of light reaching the specimen. Rhodamine blue is a fluorescent dye that, when struck with this wavelength of light, will act as a signal from the specimen to a photomultiplier tube positioned just behind a pinhole. The photomultiplier tube detects only light focused at the pinhole; light from above and below the plane of interest in the specimen is prevented by the pinhole from striking the photomultiplier tube.[67] The image is formed by recording light primarily from a small focal volume, largely ignoring points to the side or above or below. That volume, described as a point-spread function, is the product of two similar functions that are generated by the objective lens. Because of the multiplication, the recorded light is greater than even the integrated total of the light from all other points in a thick sample.[68]

The output from the photomultlplier tube is built up in a digital framestore in a microcomputer; it is displayed as an image on a video monitor screen or stored as a digital file. Images are collected at approximately one frame per second, and signal averaging is usually necessary to eliminate much of the background noise in the images.[67]

MATERIALS AND METHODS

PILOT STUDIES

Before experimentation began, several pilot studies were conducted to determine the most accurate method of extraction of the fluoride ion from its solution in the calophony-based varnish. This extraction allows the fluoride ion to be measured by direct fluoride ion analysis. Initial attempts at extracting the fluoride ion from the calophony base included dissolving a known amount of varnish in chloroform and performing a series of distilled water washes. This procedure was done in glass petri dishes. Other attempts included heating a known sample of varnish in a solution of sulfuric acid, sodium hydroxide, and TISAB II buffer. The first attempt at extracting fluoride directly into a surrounding pool of water involved placing 1.0 g of varnish into 5.0 ml of DI water.

All these early studies were conducted, and fluoride ion concentration was measured by direct fluoride analysis. The pilot study protocols were modified until a fluoride ion concentration was obtained that was similar to theoretical values (~22,600 ppm).

TOOTH SELECTION AND PREPARATION

One hundred premolar enamel specimens (3 mm in diameter) were drilled from extracted, human teeth, which were obtained from oral surgeons and disinfected in 10-percent buffered formalin (pH 6.8 to 7.0) for at least two weeks. Each specimen was mounted on a polyacrylic rod using denture acrylic and randomly coded with a three-digit

number (#000 - #099). Specimens were then divided at random between five groups. All specimens were ground using 600-grade silicon carbine paper to remove approximately 50 μm of the surface and then polished to a high luster with Gamma Alumina (0.05 μm) using standard methods. A strip of nail polish was painted on all specimens, to a width of around 1.0 mm (around 33 percent of the specimens' surface). This protected natural surface was used as a sound reference for QLF analysis.

INITIAL CARIES CHALLENGE

All of the specimens were placed individually in 14 ml of a 50-percent saturated hydroxyapatite (HAP)/0.1M lactic acid carbopol solution (pH 5.0), at 37 °C for 72 hours, so that 30- to 40-μm deep lesions developed. This caused enamel demineralization to occur at the unpainted area of each tooth. Following initial lesion formation, all teeth were rinsed and stored in humid conditions. Once again, a 1.0-mm-wide strip was painted on each specimen with fingernail polish (which is acid resistant), so that a grand total of 66 percent of each specimen's surface was now covered by polish. Thus, 33 percent of each specimen was initially covered by polish to protect the natural sound tooth, and 33 percent of each specimen was covered by polish after demineralization to protect a part of the area demineralized by the initial caries challenge (baseline lesion).

TREATMENT REGIMENS

Group A (Duraflor – horizontal): Twenty tooth specimens each had enough 5.0-percent NaF Duraflor painted on them to completely cover the remaining

unpainted third. The varnish used in this group was stored in a horizontal position one week prior to use.

Group B (Duraflor – vertical): Twenty tooth specimens each had enough 5.0-percent NaF Duraflor painted on them to completely cover the remaining unpainted third. The varnish used in this group was stored in a vertical position (cap end upwards) for one week prior to use.

Group C (Duraphat – horizontal): Twenty tooth specimens each had enough 5.0-percent NaF Duraphat painted on them to completely cover the remaining unpainted third. The varnish used in this group was stored in a horizontal position one week prior to use.

Group D (Duraphat – vertical): Twenty tooth specimens each had enough 5.0-percent NaF Duraphat painted on them to completely cover the remaining unpainted third. The varnish used in this group was stored in a vertical position (cap end upwards) for one week prior to use.

Group E (CavityShield): Ten tooth specimens each had enough 5.0-percent NaF CavityShield (from a 0.40 ml unit dose) painted on them to completely cover the remaining unpainted third.

Group F (Negative Control): Ten tooth specimens did not have any fluoride varnish placed on the remaining unpainted third. This area remained untouched and uncovered.

The contents of the four 10-ml tubes of 5.0-percent NaF Duraflor were completely dispensed from each tube in the following manner: Each tube was opened immediately before the dispensing of its varnish. Ten samples (weighing approximately 1.0 g apiece) were obtained from each 10-ml tube by means of manual squeezing. Each sample was dispensed into its own plastic dappen dish until varnish weight reached approximately 1.0 g. Thus, each 10-ml tube delivered 10 samples into 10 separate dappen dishes (creating 40 total samples of Duraflor varnish). This same procedure was repeated for the four 10-ml tubes of Duraphat varnish, thus creating in total 40 dappen dishes filled with approximately 1.0 g of Duraphat varnish apiece.

All 80 utilized dappen dishes (40 from Duraflor and 40 from Duraphat) were then treated in a similar manner: The contents of each dish were mixed with a disposable, individual bend-a-brush. This brush was then used to cover the remaining third (not covered with fingernail polish) of a tooth specimen with fluoride varnish. This same brush was then used to paint a sample of varnish at the bottom of a plastic specimen jar. The weight of this varnish in the jar was recorded and labeled, so that it was identified with its corresponding tooth specimen. All 80 plastic specimen jars were then filled with 100 ml of deionized water and allowed to sit for seven days, while occasionally being stirred. The water in these 80 jars was then analyzed for fluoride ion content using direct analysis. The varnished teeth were stored in humid conditions at 4 ^{o}C, for 20 hours. The physical barrier of the fluoride varnish resin base was then removed with a scalpel and

checked under a stereomicroscope (X10). All 100 tooth specimens were then subjected to the same caries challenge as described previously (post-treatment lesion).

The 10 unit-doses of 0.40 ml 5.0-percent NaF CavityShield were used in the following manner: Each unit dose was opened and mixed, according to manufacturer's instructions. The brush included in each unit dose was then used to cover the remaining third (not covered with fingernail polish) of each tooth specimen in this group. Each of these brushes was then used to paint a sample of varnish onto the bottom of a plastic specimen jar. The weight of the varnish sample was then recorded and labeled, so that it was identified with its corresponding tooth specimen. Each tooth specimen was allowed to dry and was stored in humid conditions at 4 ^{o}C, for 20 hours. Next, they were all subjected to the final caries challenge. The 10 specimen jars of CavityShield samples were each filled with 100 ml of deionized water and allowed to sit (with occasional stirring) for seven days. After this time period, the water in each jar was analyzed for fluoride ion content using direct fluoride analysis.

DIRECT FLUORIDE ANALYSIS

Direct analysis for fluoride was accomplished using a combination fluoride ion-specific electrode (Orion No. 96-09-00) and a pH/ion meter (Accumet 950, Fisher Scientific, Cincinnati, OH, USA). The fluoride extract collected from each of the plastic specimen jars was diluted with TISAB II buffer in a ratio of 1:1 and placed directly under the electrode, resulting in a millivolt (mv) measurement. Each assay was duplicated to measure reproducibility. Fluoride content was determined by comparison with a series of known standards similarly analyzed at the same time. This was done for all 80 samples.

QLF ANALYSIS

All 100 teeth were analyzed by the QLF system, to measure the amount of demineralization. Prior to QLF analysis, the transparent acid-resistant nail polish was carefully removed using acetone. Images of all specimens' windows were taken using the QLF system (QLF/clin 007, Inspektor Research Systems F.V., The Netherlands). Briefly, this system consists of a lamp unit and a camera control unit, which is connected via a liquid light guide and electrical cable to a camera handpiece. Specimens were exposed to 13 mW/cm^2 of violet-blue light (wavelength: 290-450 nm) via the liquid light guide in the camera handpiece. Images were captured through a 510 nm band-pass filter, using a miniature CCD camera located inside the handpiece. Images were stored in the Quantitative Light-induced Fluorescence 1.96w (QLF) program (Figures 1, 2, and 3). Then, images were analyzed by comparing the fluorescence values of pixels from sound (sound specimen area) and demineralized areas (either baseline lesion or post-treatment lesion areas). In order to calculate the fluorescence loss, a computer-generated rectangle (inner patch) was placed in the area of analysis. The size of the rectangle was kept constant for all the analyses. The fluorescence values of the pixels within the rectangle were compared with the fluorescence values of the pixels surrounding the rectangle. In this study, the QLF program was set to define tissue as demineralized if its fluorescence is 95 percent or less than the fluorescence values of the surrounding sound enamel (indicating that fluorescence values at less than 95 percent of their sound reference fluorescence values were considered demineralized). The software provided the average and maximum percentage of fluorescence loss for each area analyzed. ΔQ was calculated

as the average change of fluorescence multiplied by the lesion area. Both the baseline lesion and post-treatment lesion areas were analyzed in the same manner. Then, in order to obtain the treatment (fluoride varnish) effect for each specimen, the difference between the post-treatment data minus the baseline lesion data was calculated. A negative value resulting from this subtraction indicated more demineralization had occurred, while a positive number indicated remineralization.

CONFOCAL MICROSCOPY ANALYSIS

After final QLF analysis, all specimens were cut in half, so that each half contained a sound area, an initial lesion area, and a post-treatment lesion area. One of these halves from every specimen was placed into storage at 4 °C in humid conditions. The other half from every specimen was stained with a freshly prepared 0.1-mM Rhodamine B solution (Aldrich Chem. Co. Milwaukee, WI, USA) overnight, without further rinsing. Then, the stained demineralized areas of each specimen were analyzed for depth, area, and total lesion fluorescence. The cut, stained surface of each specimen was allowed to dry before being analyzed with the confocal laser scanning microscope (Odyssey, Noran Instruments, Inc., Middleton, WI, USA) to determine the extent of the lesions.[63] In this study, the samples were examined using Image 1 (Version 4.14.C) software (Universal Images Corp., West Chester, PA). After being brought into focus (using an X10 Nikon objective, N.A. 0.25), the specimens were illuminated with an ion argon laser using a 488-nm excitation wavelength. Confocal slits were set at 10 μm with a 515-nm long-pass barrier filter, and the argon laser intensity was set at 100 percent. For collection of the images, samples were frame-averaged using 128 frames per image

(Figures 4, 5, and 6). Measurements were made in the same two areas that were analyzed by QLF (baseline lesion area and post-treatment area). Areas were scanned planoparallel to the transversal cut surface of the specimen and perpendicular to the natural surface of the tooth. Differences in each of the measured confocal paramenters between baseline and treated lesions were determined for each specimen. In this case, a negative value indicated remineralization, while a positive value indicated lesions had progressed after treatment.

STATISTICAL METHODS

Fluoride Ion Concentration

The five groups (Duraphat horizontal storage, Duraflor horizontal storage, Duraphat vertical storage, Duraflor vertical storage, and CavityShield) were compared for differences in absolute mv and fluoride μg/gm using one-way analysis of variance (ANOVA). Pairwise comparisons between the groups were made using Tukey's multiple comparisons procedure to control the overall significance. Comparisons were considered to be statistically significant if the p-value was less than 0.05. The order effect on the Duraphat and Duraflor groups was examined using repeated measures ANOVA. Repeatability of the mv measurements was assessed using a paired t-test and an intraclass correlation coefficient (ICC).

Quantitative Light Fluorescence (QLF)

The Duraphat and Duraflor groups were compared for differences in ΔQ using two-way ANOVA. Pairwise comparisons between the groups were made using Tukey's

multiple comparisons procedure to control the overall significance. Comparisons were considered to be statistically significant if the p-value was less than 0.05.

Confocal Microscopy

The difference between the demineralization and remineralization confocal measurements was computed as: (demin – remin). The six groups (Duraphat horizontal storage, Duraflor horizontal storage, Duraphat vertical storage, Duraflor vertical storage, CavityShield, negative control) were compared for differences in confocal depth, area, and total fluorescence using one-way analysis of variance (ANOVA). The Duraphat / Duraflor and storage effects were compared using two-way ANOVA. Pairwise comparisons between the groups were made using Tukey's multiple comparisons procedure to control the overall significance. Comparisons were considered to be statistically significant if the p-value was less that 0.05. The effect of order on the Duraphat and Duraflor groups was examined using repeated measures ANOVA.

RESULTS

PILOT STUDIES

Every attempt at extracting fluoride from its colophony base with chloroform resulted in a consistently low fluoride ion concentration, regardless of what type of varnish was used. Typical readings were from 1500 ppm to 1900 ppm. Attempts at using the acid/NaOH/buffer solution resulted in similar results. Placing 1.0 g of varnish in 5.0 ml of deionized water followed by immediate direct fluoride analysis resulted in unmeasurable mv readings.

FLUORIDE CONCENTRATION

Repeatability

The intraclass correlation coefficient (ICC) for assessing agreement between the repeated mv measurements was 0.92, indicating good repeatability. However, the second reading was consistently lower than the first ($p = 0.0001$). All results used for statistical analysis for absolute mv were obtained from the first measurement.

Absolute mv

Duraphat horizontal storage had significantly lower absolute mv than Duraflor horizontal storage ($p = 0.0426$) and Duraflor vertical storage ($p = 0.028$). Duraphat vertical storage had significantly lower absolute mv than Duraflor horizontal storage ($p = 0.0445$) and Duraflor vertical storage ($p = 0.0030$). No other significant differences were found between the groups ($p > 0.18$) (Table I). The order effect was significant for

Duraflor ($p < 0.05$): the #10 sample was significantly different from #1, #3, #4, #5, #6. The order effect was not significant for Duraphat ($p = 0.99$).

Fluoride μg/gm

There were no significant fluoride μg/gm differences between the groups ($p = 0.29$). (Table II). The order effect was significant for Duraflor (stored both horizontally and vertically) ($p < 0.05$): the #10 sample was significantly different from all others, and the #9 sample was significantly different from #1, #5, #6, #7, #8. (Figures 7 and 8; mean ppm Duraflor horizontal and vertical, Table III). The order effect was not significant for Duraphat ($p = 0.99$). (Figures 9 and 10; mean ppm Duraphat horizontal and vertical, Table IV).

QLF

There was no significant difference on ΔQ scores due to storage method ($p = 0.81$) or product used ($p = 0.87$). All groups showed remineralization (shown by a positive mean value) except the negative control (shown by a negative mean value) (Table V).

CONFOCAL MICROSCOPY

Confocal Diameter (Depth of Lesion)

All groups were significantly different from the negative control ($p < 0.0020$). The fluoride-treated groups showed remineralization (shown by a negative mean value), while the negative control group showed more demineralization. Duraphat and Duraflor

were significantly different for vertical storage ($p = 0.0411$). No other significant differences were found between groups ($p > 0.31$) (Table VI). The order effect was not significant ($p = 0.62$).

Lesion Area

All groups were significantly different from the negative control ($p < 0.0020$). Duraphat and Duraflor were significantly different for vertical storage ($p = 0.0409$). No other significant differences were found between groups ($p > 0.19$) (Table VII). The order effect was not significant ($p = 0.96$).

Total Lesion Fluorescence

All groups were significantly different from the negative control ($p = 0.0001$). No other significant differences were found between groups ($p > 0.81$) (Table VIII). The order effect was not significant ($p = 0.99$).

TABLES AND FIGURES

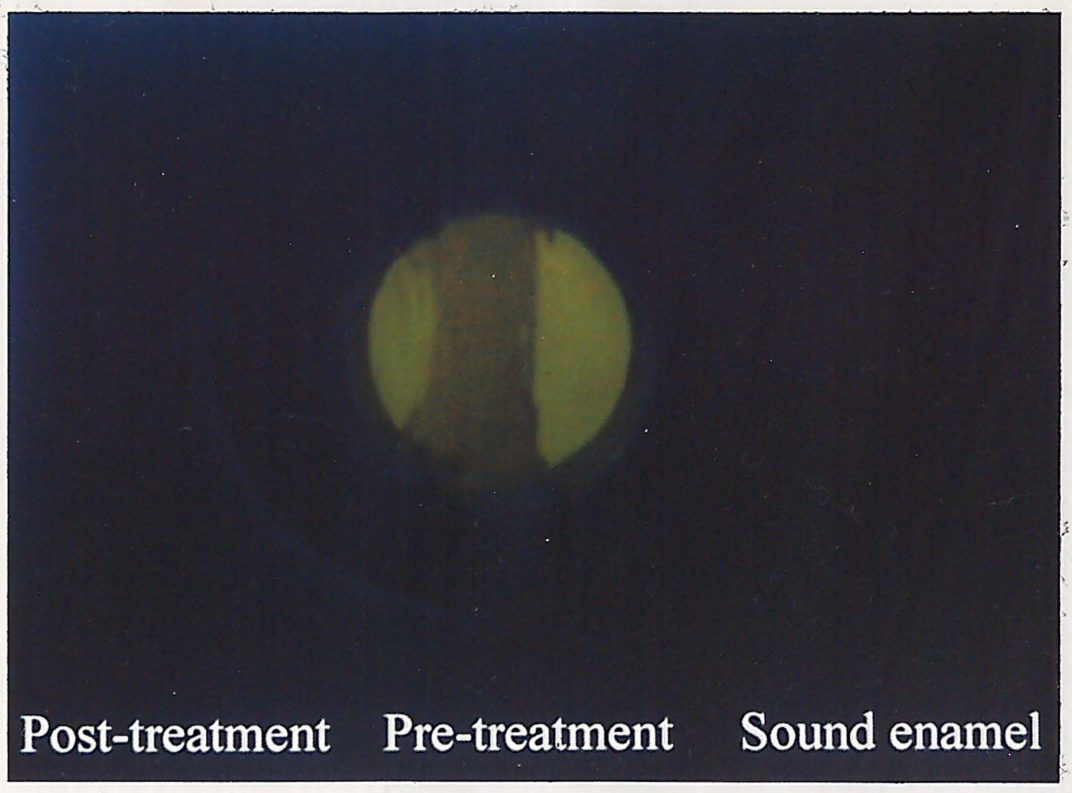


FIGURE 1. QLF image with post-treatment lesion showing large amounts of remineralization.

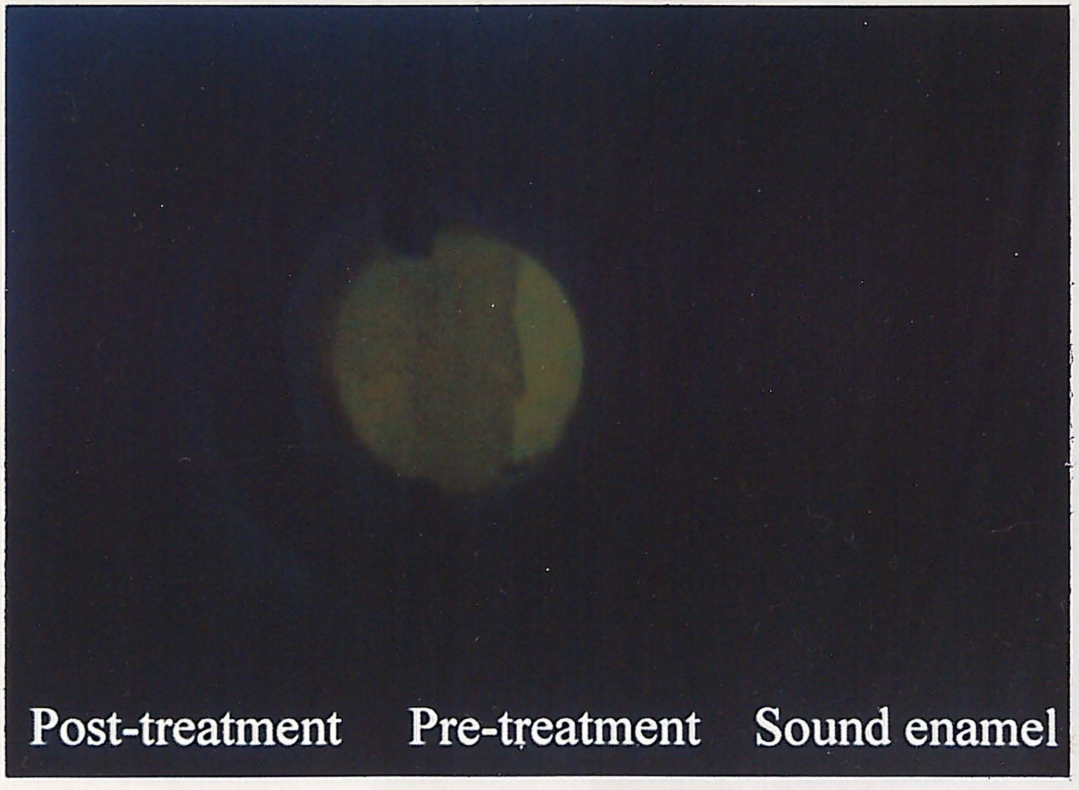


FIGURE 2. QLF image with post-treatment lesion showing moderate amounts of remineralization.

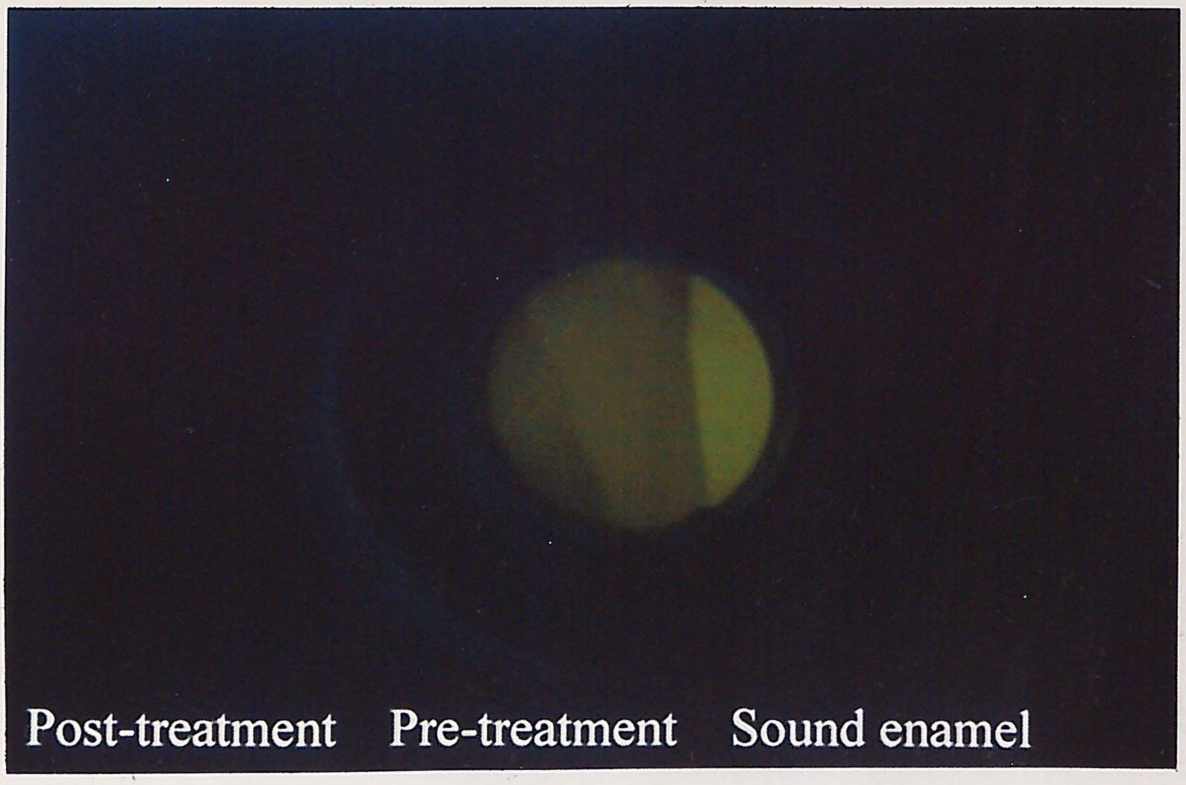


FIGURE 3. QLF image with post-treatment lesion showing progression of demineralization.

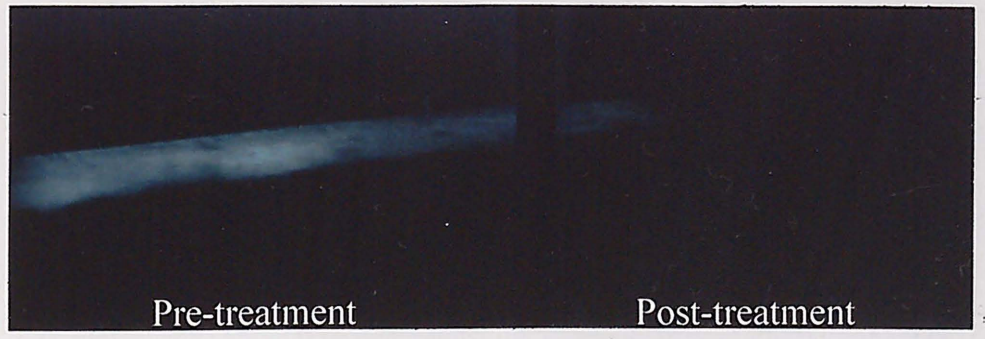


FIGURE 4. Confocal images with post-treatment lesion showing large amounts of remineralization.

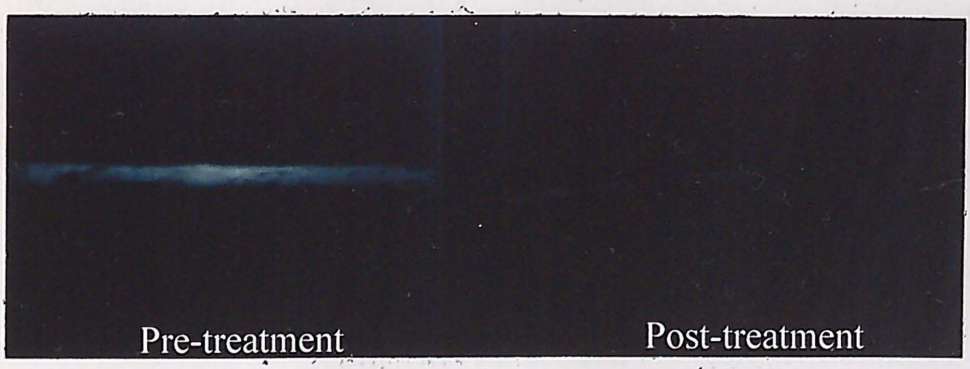


FIGURE 5. Confocal images with post-treatment lesion showing moderate amounts of remineralization.

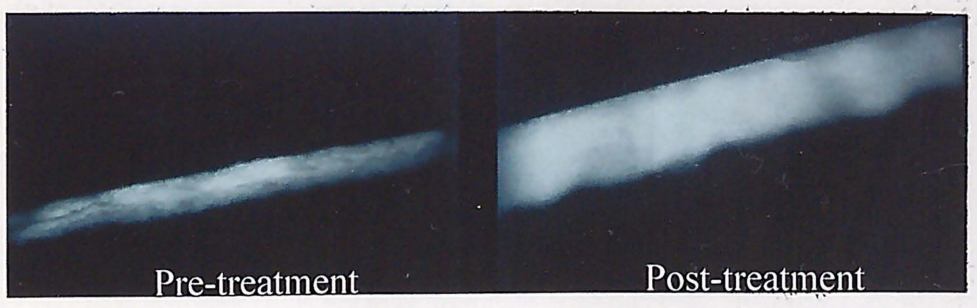
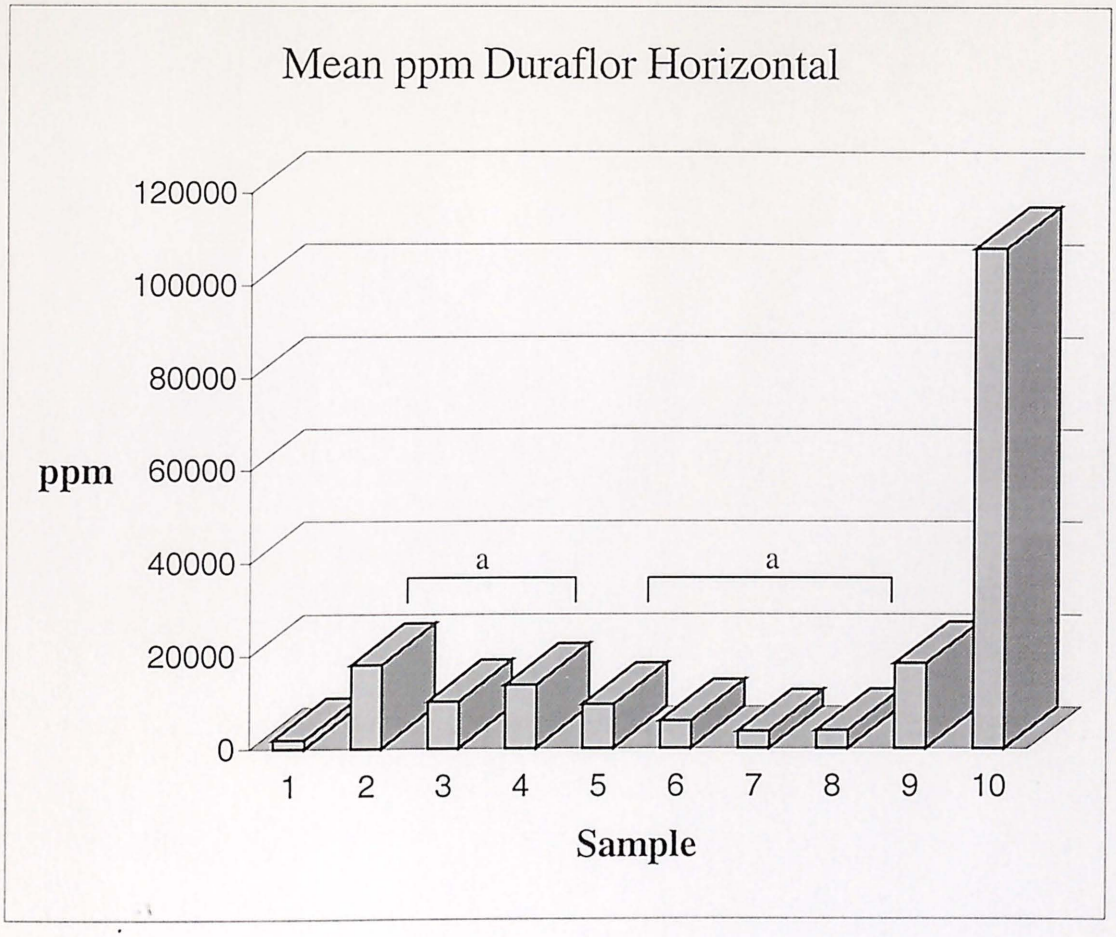
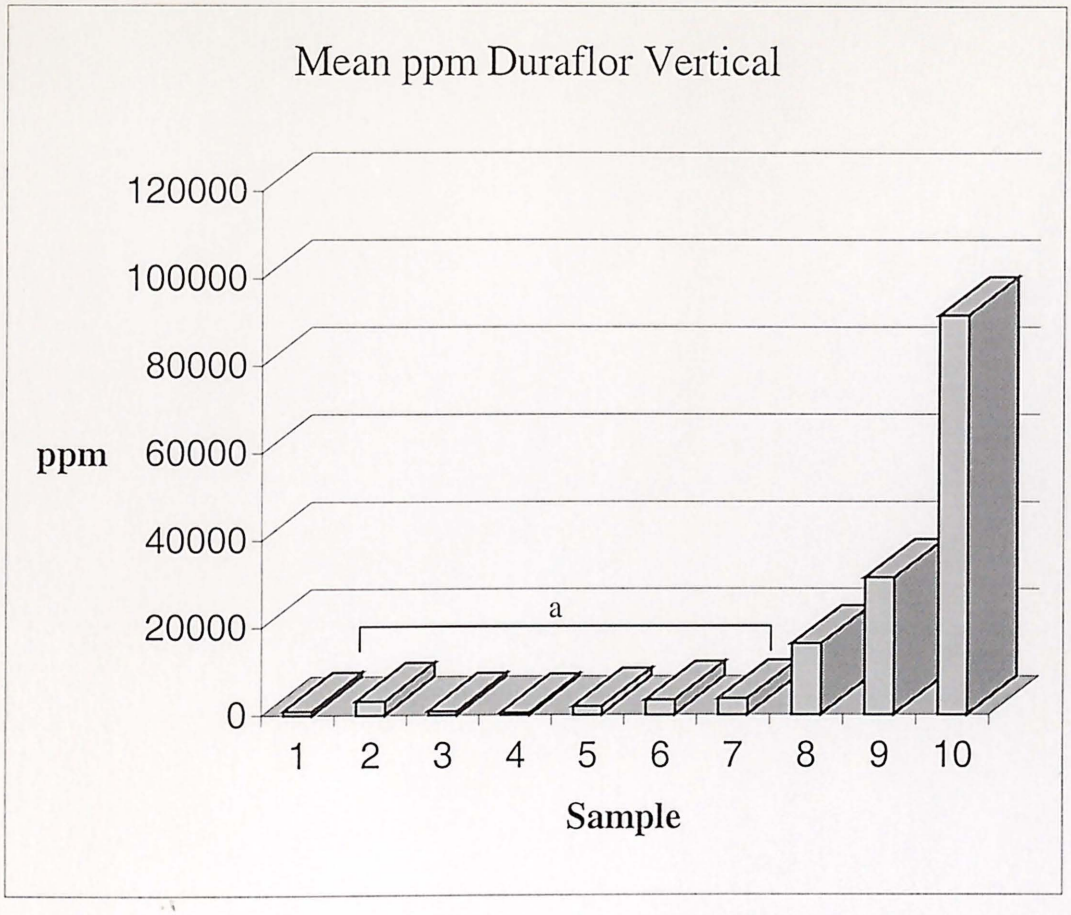


FIGURE 6. Confocal images with post-treatment lesion showing progression of demineralization.



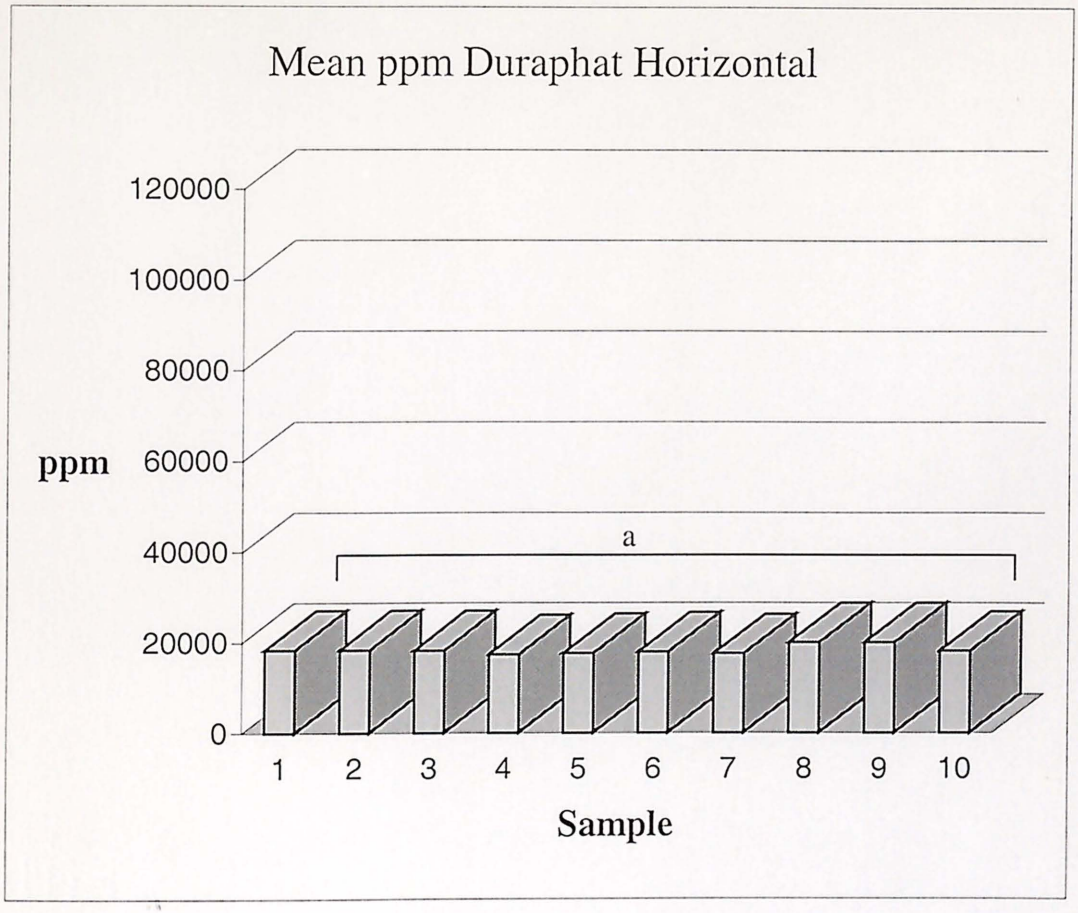
a- groups within brackets are not significantly different ($p>0.05$)

FIGURE 7. Mean ppm fluoride concentrations from horizontally stored tubes of Duraflor. Sample #1 represents the first ml dispensed from the tube, sample #10 is the last ml dispensed from the tube.



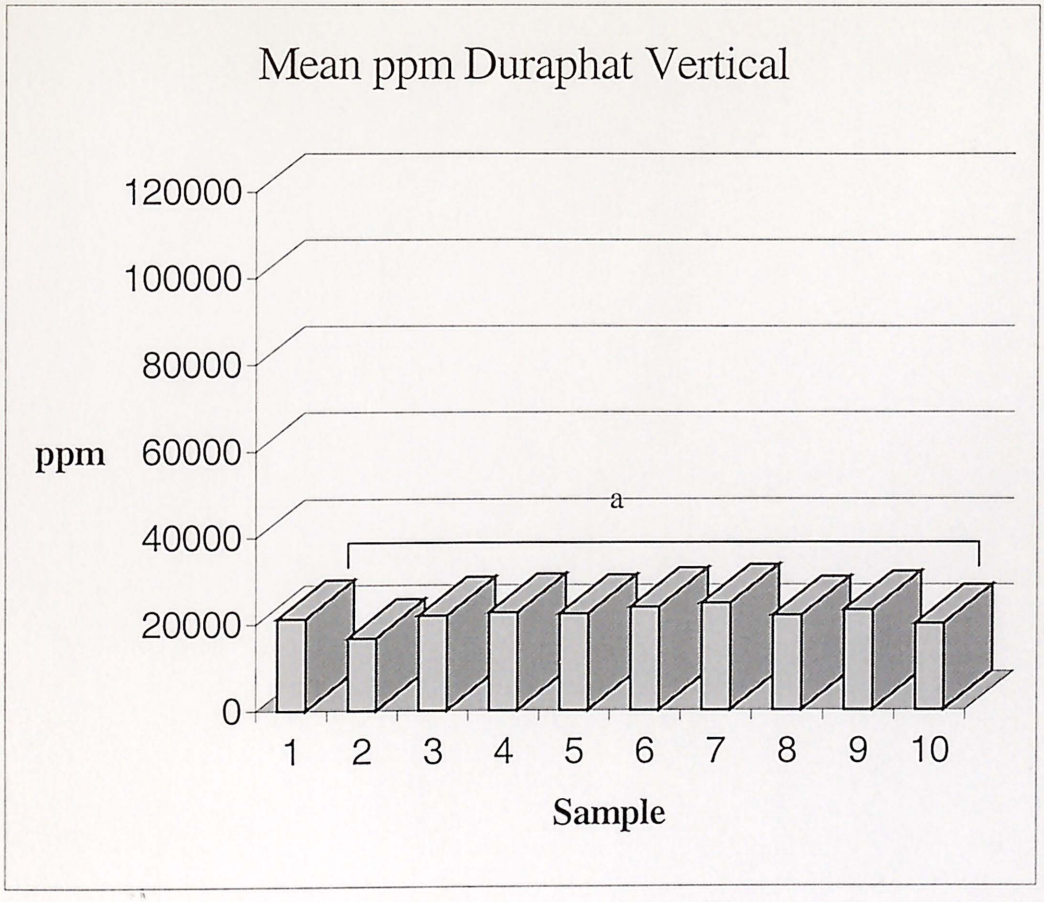
a – groups within brackets are not significantly different ($p>0.05$)

FIGURE 8. Mean fluoride concentrations from vertically stored tubes of Duraflor. Sample #1 represents the first ml dispensed from the tube, sample #10 is the last ml dispensed from the tube.



[a]Groups within brackets are not significantly different ($p > 0.05$).

FIGURE 9. Mean fluoride concentrations from horizontally stored tubes of Duraphat. Sample #1 represents the first ml dispensed from the tube; sample #10 is the last ml dispensed from the tube.



[a]Groups within brackets are not significantly different ($p > 0.05$).

FIGURE 10. Mean fluoride concentrations from vertically stored tubes of Duraphat. Sample #1 represents the first ml dispensed from the tube; sample #10 is the last ml dispensed from the tube.

TABLE I

Absolute mv measured by
direct fluoride analysis

Product	N	Mean		SD	Min	Max
Duraphat Horizontal	20	55.6	a	3.8	49.6	63.2
Duraphat Vertical	20	55.7	a	4.8	47.1	65.2
CavityShield	10	82.4		2.9	76.8	86.5
Duraflor Horizontal	20	84.0	a	46.2	4.2	163.1
Duraflor Vertical	20	93.1	a	47.7	0.9	157.5

[a]Groups within brackets are not significantly different ($p > 0.05$).

TABLE II

Fluoride μg/gm (ppm) measured
by direct fluoride analysis

	Product	N	Mean	SD	Min	Max
a	Duraphat Vertical	20	21838	2908	15346	25399
	Duraflor Horizontal	20	19331	32588	88	124174
	Duraphat Horizontal	20	18387	3096	11439	24488
	Duraflor Vertical	20	15333	27990	113	99223
	CavityShield	10	4992	968	3251	6616

[a]Groups within brackets are not significantly different ($p > 0.05$).

TABLE III

Mean ppm fluoride concentrations from horizontally and vertically stored tubes of Duraflor

Storage	Order	N	Mean	SD	Min	Max
Horizontal	1	2	2003	982	1308	2698
a	2	2	18127	25511	88	36165
a	3	2	10100	14093	135	20065
a	4	2	13713	18413	693	26733
a	5	2	9455	7267	4317	14594
a	6	2	5871	1771	4618	7123
a	7	2	3699	1351	2744	4655
a	8	2	3835	616	3400	4271
	9	2	18372	16671	6584	30160
	10	2	108137	22680	92100	124174
Vertical	1	2	892	573	487	1297
a	2	2	3135	2956	1044	5226
a	3	2	797	422	499	1095
a	4	2	520	576	113	927
a	5	2	1812	1523	735	2888
a	6	2	3359	90	3295	3423
a	7	2	3649	1196	2803	4495
	8	2	16172	5585	12223	20121
	9	2	31395	8524	25367	37422
	10	2	91599	10781	83975	99223

[a]Groups within brackets are not significantly different ($p > 0.05$).

TABLE IV

Mean ppm fluoride concentrations from horizontally and vertically stored tubes of Duraphat

Storage	Order	N	Mean	SD	Min	Max
Horizontal	1	2	18367	295	18158	18576
	2	2	18410	4300	15369	21450
	3	2	18281	3827	15575	20987
	4	2	17382	1980	15982	18782
a	5	2	17688	3122	15480	19896
	6	2	17963	9227	11439	24488
	7	2	17558	3229	15275	19842
	8	2	20045	2643	18176	21914
	9	2	20037	1946	18661	21413
	10	2	18143	3616	15586	20700
Vertical	1	2	21272	2545	19473	23072
	2	2	16796	2051	15346	18246
	3	2	21871	3704	19251	24490
	4	2	22599	402	22314	22883
a	5	2	22319	3304	19983	24655
	6	2	23868	130	23776	23960
	7	2	24675	1023	23952	25399
	8	2	21897	4379	18801	24994
	9	2	23120	268	22930	23309
	10	2	19965	4144	17035	22895

[a]Groups within brackets are not significantly different ($p > 0.05$).

TABLE V

QLF: ΔQ caries data (post-treatment minus baseline data)[a]

	Product	N	Mean	SD	Min	Max
b	Duraphat Vertical	20	6005.5	8163.7	-8126.3	19437.6
	Duraflor Vertical	20	4480.4	9833.0	-11698.3	21164.1
	Duraflor Horizontal	20	4438.0	8394.4	-12055.5	19684.5
	Duraphat Horizontal	19	4168.9	10604.8	-16074.0	26177.1
	CavityShield	9	4167.0	11853.4	-6608.2	33660.4
	Negative Control	10	-4488.6	7256.7	-12859.2	10423.7

[a]A positive number indicates remineralization. A negative value indicates lesion progression.
[b]Groups within brackets are not significantly different ($p > 0.05$).

TABLE VI

Confocal caries lesion depth (post-treament minus baseline data)[a]

	Product	N	Mean	SD	Min	Max
	Duraflor Vertical	20	-16.75	16.26	-56.89	2.54
b	Duraphat Horizontal	19	-11.36	18.72	-39.77	21.79
b	Duraflor Horizontal	19	-7.81	11.76	-27.13	20.77
b	CavityShield	10	-5.49	9.20	-19.67	6.96
	Duraphat Vertical	20	-1.34	23.13	-49.97	53.42
	Negative Control	10	25.23	17.55	0.00	50.63

[a]A negative number indicates remineralization. A positive number indicates lesion progression.
[b]Groups within brackets are not significantly different ($p > 0.05$).

TABLE VII

Confocal lesion area (post-treatment minus baseline data)[a]

	Product	N	Mean	SD	Min	Max
	Duraflor Vertical	20	-5218	5021	-16860	1000
b	Duraphat Horizontal	19	-3948	6303	-14007	11843
b	CavityShield	10	-3703	4934	-13711	2900
b	Duraflor Horizontal	19	-2860	4841	-12237	4680
	Duraphat Vertical	20	-38	7575	-17723	16520
	Negative Control	10	9379	5891	0	16890

[a]A negative value indicates remineralization. A positive value indicates lesion progression.
[b]Groups within brackets are not significantly different ($p > 0.05$).

TABLE VIII

Confocal total lesion fluorescence (post-treatment minus baseline data)[a]

	Product	N	Mean	SD	Min	Max
b	Duraphat Horizontal	19	-558730	986570	-2346500	1341100
b	Duraflor Vertical	20	-437155	862383	-2948000	560700
b	CavityShield	10	-304850	623194	-1826100	362000
b	Duraflor Horizontal	19	-221511	847339	-1760700	2050600
b	Duraphat Vertical	20	-175910	978501	-1796000	1869200
	Negative Control	10	1700810	1415827	0	4134000

[a]A negative value indicates remineralization. A positive value indicates lesion progression.
[b]Groups within brackets are not significantly different ($p > 0.05$).

DISCUSSION

Several pilot studies were conducted prior to experimentation. The purpose of the studies was to determine the best method of extracting fluoride ion from a varnish solution resulting in the most accurate (closest to theoretical value) reading with a fluoride ionometer (direct fluoride analysis). A 5.0-percent sodium fluoride varnish contains 50 mg of NaF per ml of varnish. Specifically, one ml of varnish contains 22.6 mg of fluoride ion. The standard abbreviation ppm (parts per million) for fluoride represents the number of μg of fluoride ion per g (or ml) of solute. Therefore, a solution of 5.0-percent NaF should theoretically contain 22,600 ppm fluoride ion. Early attempts at extracting fluoride ion out of a varnish solution involved using chloroform to dissolve the calophony-based varnish, followed by serial extractions of deionized water. In these attempts, the varnish solutions were stored in glass flasks. However, repeated results showed fluoride was recovered at concentrations from 1500 ppm to 1900 ppm. It was concluded that dissolving the fluoride varnishes in chloroform affected fluoride ion release in such a way that not all available ions were extracted from solution. It was also noted that glass containers should not be used to hold ion-containing solutions, because the fluoride ion will bind to the glass, resulting in skewed results.

Further pilot studies were conducted, with all solutions being held in plastic containers. It was theorized that because fluoride ion is released from its colophony-based varnish intraorally, fluoride ion should be able to be extracted over time in an aqueous setting. Early attempts to test this theory involved placing 1 g of varnish in 5 ml of water, letting the solution sit for seven days, and then using direct fluoride analysis.

Results from these attempts yielded unmeasurable millivolt readings on the ionometer. Because the concentration of fluoride in 5.0-percent NaF varnishes is so great, it was noted that a much smaller amount of varnish would have to be placed in a much larger amount of deionized water. These measurements were modified until a ppm value close to 22,600 ppm was obtained. In the end, an average of 0.030 g of varnish was placed in 100 ml of deionized water to achieve the theoretical values. This same protocol for extracting fluoride ion of its varnish solution is now being used at other universities for similar purposes.

One purpose of this study was to measure the fluoride concentration gradient in 10-ml tubes of fluoride varnish, based on the resting position of the tube prior to use. It was theorized that the resting position of a tube will create a wide fluoride ion concentration gradient. However, it was found that regardless of storage position and which part of the tube the varnish came from, Duraphat tubes consistently provided varnish with a fluoride concentration similar to theoretical values. In essence, no concentration gradient exists within tubes of Duraphat. On the other hand, Duraflor varnish does seem to have a fluoride ion concentration gradient, not only based on its resting position, but perhaps due to the way each tube is mechanically filled with varnish. A tube of Duraflor stored horizontally has an even fluoride ion concentration for the first 9 ml dispensed, with some values close to theoretical values. However the last 1.0 ml dispensed from these tubes consistently had fluoride concentration values close to 100,000 ppm. This leads one to believe that this bolus of fluoride may be trapped at the end portion of a tube after manufacturing. A future study to help solve this problem would entail storing the tubes three ways: horizontally, cap end up, and cap end down.

A tube of Duraflor stored vertically with its capped end up appears to house a fluoride ion concentration gradient. Fluoride concentration readings from the first 4 ml dispensed were very low, around the range of 700 ppm. This value is less than most fluoride-containing dentifrices (1100 ppm) in the U.S. As varnish from the last half of the tube was measured, it was noted that the fluoride concentration consistently increased from ~3400 ppm up to ~92,000 ppm. Therefore, it is concluded that a fluoride ion concentration does exist in tubes of Duraflor based on resting position.

These findings have several clinical implications. First and foremost is the thought of fluoride toxicity in children. Fluoride varnish contains the highest fluoride concentration of any vehicle. It has been reported by Cameron and Widmer[37] that gastrointestinal symptoms were noted in children after ingestion of 3 to 5 mg fluoride/kg. Fatalities have been documented of children who ingested fluoride at doses of 16 mg fluoride/kg. According to manufacturers, each dose of fluoride varnish given per patient should only be from 0.3 to 0.5 ml. At a theoretical value of 22,600 ppm, a 0.5 ml dose of varnish contains 11.3 mg of fluoride ion. Using a toxic dose of 3 mg fluoride/kg body weight, a 20 kg (44 pound) child would need to ingest 60 mg of fluoride to accrue symptoms. One 0.5 ml dose of varnish does not come close to this level. However, when treated with 0.5 ml of varnish with a concentration of 100,000 ppm (as seen in some doses of both storage methods of Duraflor), a child comes much closer to the toxic level of 60 mg. Zero-point-five ml of varnish at this concentration contains 50 mg of fluoride ion. A full milliliter of this varnish would obviously contain 100 mg, well above the toxic dose. To reach a fatal dose (16 mg fluoride/kg), a 20 kg child would need to ingest 6.4 ml of varnish containing 100,000 ppm fluoride. The chances of this occurring

seem unlikely but are noteworthy. No child should ever be left alone in an operatory for any reason with such potential hazards as high-dosed fluoride varnishes made available to them. The hazard of fluoride toxicity/accidental ingestion has led many to begin using unit-dosed packaged fluoride varnishes, such as CavityShield. This brand of fluoride varnish is available in either a 0.25-ml package or a 0.4-ml package. The theoretical total amount of fluoride in a 0.25-ml package is 5.65 mg, and in a 0.4-ml package the total is 9.05 mg fluoride. In the current study, recovered concentrations of fluoride from CavityShield were lower than the theoretical value. Perhaps mixing the varnish better within its mixing well would result in a higher ppm value.

Unless each dose is weighed chairside, it is impossible to measure the exact amount of fluoride varnish dispensed per dose given to a child from a 10-ml tube. Duraphat varnish is much more viscous than Duraflor, but neither is quantifiable when dispensed into a dappen dish or onto a mixing pad. Likewise, it is thus impossible to determine how much fluoride is placed onto a child's teeth. Thus, when manufacturers recommend to place between 0.3 to 0.5 ml of varnish on a child's teeth, only guesswork from a dentist's perspective will determine how much fluoride is actually placed on teeth. Regarding toxicity and cost-effectiveness, these are reasons why more dentists are switching to a unit-dosed fluoride varnish, such as CavityShield.

This study also had the purpose of comparing and contrasting the caries inhibition properties of Duraphat, Duraflor, and CavityShield, and to determine whether a fluoride concentration gradient affected these properties. Using confocal microscopy as the gold standard, it was found that all three brands of varnish, regardless of how they were stored, and from what part of the tube a sample came, were able to inhibit an *in vitro* caries

process. On average, remineralization of enamel occurred in every treatment group, whereas lesion progression occurred in the negative control group. This is clinically relevant for those using Duraflor varnish in that even though a large concentration gradient exists within tubes, the *in vitro* caries process can still be halted. However, it was noted numerically that the greater the fluoride concentration used to treat a tooth, the greater the remineralization process. The differences were not statistically significant, probably because of the small sample sizes used. Whether these numerical differences are clinically relevant remains to be proved. It can thus be concluded that the benefits from treating a tooth with the first half of a tube of vertically stored Duraflor may not last as long as a tooth treated with a higher concentration of fluoride. Further studies are needed to investigate this matter.

A final purpose of this study was to determine if Quantitative Light-Induced Fluorescence (QLF) can detect differences in lesions developed when exposed to an artificial caries environment and fluoride varnish. It was hypothesized that QLF does have this ability. After data analysis, it was noted that the ΔQ (described earlier) of treated lesions versus non-treated lesions was not statistically significant. However, it is noted that mean values of every treatment group indicate enamel remineralization and that the mean value of untreated lesions indicate lesion progress (further demineralization). The differences between all groups were not statistically significant, likely due to the small sample sizes used. It may thus be concluded that QLF is a very effective technique that is able to detect and monitor early enamel lesions. Clinically, this is important if the operator is able to use a handheld QLF intraoral device and detect early lesions before cavitation. Then, treatment of these early lesions with topical

fluorides may be monitored to prevent future demineralization without the need for operative cavity preparation and restoration.

SUMMARY AND CONCLUSIONS

Numerous studies have been performed showing the clinical and *in vitro* efficacy of fluoride varnish as an anti-caries agent. Recently, it has been shown that separation of contents exists within 10-ml tubes of fluoride varnish. Also, in an effort to detect early areas of demineralization, promising research is currently being conducted in the use of quantitative light-induced fluorescence (QLF). The current study had several purposes: 1) to measure the fluoride concentration gradient in these 10-ml tubes of varnish, based on resting position of the tube prior to use; 2) to compare a varnish's concentration gradient to its ability to inhibit caries in an artificial caries environment; 3) to compare and contrast the fluoride concentration gradients and caries inhibition properties of three fluoride varnishes on the American market (Duraphat, Duraflor, and CavityShield); and finally 4) to determine if QLF can detect differences in lesions developed when exposed to an artificial caries environment and fluoride varnish.

Tubes of Duraphat and Duraflor (stored in different positions prior to experimentation) were dispensed into 10 equal parts and analyzed for fluoride ion content. Human teeth specimens were prepared and treated with the varnish from all 10 parts of the tubes. Several specimens were also treated with a unit-dose of CavityShield. All teeth were subjected to an artificial caries challenge before and after treatment and examined by QLF. Finally, all teeth were examined by confocal microscopy.

Results show that a fluoride ion concentration gradient does exist in tubes of Duraflor, regardless of how it is stored. However, storing a tube of Duraflor vertically creates a larger gradient than does storing it horizontally. Tubes of Duraphat were not

found to contain any fluoride ion gradient. All three brands of varnish, regardless of how they were stored and from what part of the tube the varnish was taken from, were able to remineralize incipient *in vitro* caries lesions (as detected by confocal microscopy). QLF was able to detect early caries and remineralized enamel in the specimens.

It may be concluded that a wide range of fluoride concentation may be seen in tubes of Duraflor, regardless of from which portion of the tube varnish is taken. Clinical questions regarding fluoride toxicity may be related to such high concentrations of fluoride in some doses of the Duraflor varnish. Duraphat seems to contain a rather consistent concentration (around its theoretical value) of varnish throughout the tube. CavityShield varnish yielded consistently low readings of fluoride concentration using this method. However, all three brands remineralized incipient lesions. Finally, QLF is a promising technique that is able to detect and monitor early caries.

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ABSTRACT

FLUORIDE VARNISH CONCENTRATION GRADIENT EFFECTS MEASURED BY QUANTITATIVE LIGHT FLUORESCENCE

by

Chad O. Hazelrigg

Indiana University School of Dentistry
Indianapolis, IN

Two of the three fluoride varnishes sold on the American market today are sold in 10-ml tubes of 5.0-percent NaF varnish (Duraphat and Duraflor). Pilot studies have shown that a separation of contents within these tubes exists. The purpose of the current study was four-fold: 1) to measure the fluoride concentration gradient in 10-ml tubes of fluoride varnish, based on the resting position of the tube prior to use; 2)to compare a varnish's concentration gradient to its ability to inhibit caries in an artificial caries environment; 3) to compare and contrast fluoride concentration gradients of Duraphat and Duraflor; and finally, 4) to determine if Quantitative Light Fluorescence(QLF) can detect differences in lesions developed when exposed to an artificial caries environment and fluoride varnish. Human teeth specimens were subjected to a caries challenge and treated with a sample of fluoride varnish from one of five categories: Duraphat stored horizontally and vertically for one week; Duraflor stored horizontally and vertically for one week; or a CavityShield 0.4 ml unit-dose. Results show that no significant fluoride/ppm differences exist between groups ($p = 0.29$). It was shown that the order in

which Duraflor varnish was dispensed from the tubes significantly affected the fluoride concentration ($p < 0.05$). The order effect was not significant for Duraphat ($p = 0.99$). QLF data analysis shows there is no significant difference ($p > 0.05$) in the amount of remineralization obtained by using any varnish stored in any position. This was confirmed using confocal microscopy. These results indicate that all three brands of fluoride varnish are able to remineralize incipient *in vitro* carious lesions, regardless of from which part of the 10-ml tube the varnish is taken. However, a fluoride concentration gradient exists in tubes of Duraflor. Also, QLF is able to detect demineralized and remineralized incipient lesions.

CURRICULUM VITAE

Chad Owen Hazelrigg

June 27, 1973	Born in Indianapolis, Indiana
May 1995	BS Purdue University West Lafayette, Indiana
May 1999	DDS Indiana University School of Dentistry, Indianapolis, Indiana
July 1999 to June 2001	Pediatric Dentistry Residency Indiana University School of Dentistry, Indianapolis, Indiana

Professional Organizations

American Dental Association
American Academy of Pediatric Dentistry
Indiana Dental Association